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(54) **Inclusion complexes of prostacyclin and its derivatives, their preparation and pharmaceutical compositions containing them**

(57) Prostacyclin and prostacyclin derivatives form inclusion complexes with cyclodextrins. The complexes may be prepared by contacting an aqueous solution of the cyclodextrin with prostacyclin or a derivative thereof, preferably in organic solvent solution.

The inclusion complexes have an improved stability, especially in aqueous solution, compared to the uncomplexed compounds and are extremely potent in inhibiting thrombus formation and inducing the disaggregation of thrombi already formed. The complexes can be formulated into pharmaceutical compositions in conventional manner.

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SPECIFICATION

Inclusion complexes of prostacyclin and its derivatives, their preparation and pharmaceutical compositions containing them

This invention relates to inclusion complexes of prostacyclin and its derivatives, their preparation and pharmaceutical compositions containing them.

Prostacyclin and its derivatives are effective to inhibit platelet aggregation and potentially can be used as anti-thrombotic agents. Due to their instability in aqueous solutions, however, up to the present time these compounds have not been used for clinical purposes. This invention provides new inclusion complexes of prostacyclin and its derivatives with cyclodextrins, which are stable also in aqueous solutions and have a prolonged action. The present invention further provides a method for the preparation of these inclusion complexes.

In the first step of the biosynthesis of prostaglandin starting from arachidonic acid, in an enzymatic process endoperoxides — designated as PGG_2 and PGH_2 — are formed. These endoperoxide compounds are extremely unstable and have a half-life of the order of several seconds. When the platelets collide with the vessel-walls, from the platelets these endoperoxides are released, which are then converted into prostacyclin by means of an enzyme produced by the vessel-walls. When a vessel is injured and does not produce the enzyme which converts the endoperoxides to prostacyclin, they are transformed to thromboxane which accelerates the aggregation of platelets and leads to thrombosis.

Thrombosis takes first place in the mortality statistics of young and middle-aged people all over the world. At present there is no really effective method available for the desaggregation of the thrombi already formed and for the prevention of lethal thrombus formation. An effective drug for the desaggregation of thrombi might play a life-saving role in the case of heart attacks, infarcts or cardiac thromboses by preventing the further development of a thrombus already present and inducing the desaggregation thereof. Such a drug could also be used for example to prevent pre- and post-operative thromboses.

Prostacyclin (PGI_2) chemically is (5Z) - 9 - desoxy - 6,9α - epoxy - Δ^5 - prostaglandin - $F_{1\alpha}$. It has been observed that this compound is extremely potent in inhibiting platelet aggregation and removing the thrombi already formed. Until now, however, its practical utilization seemed to be impossible since its biological half-life is very short (about 2 minutes). In aqueous solution the compound is substantially decomposed in about 4 minutes. The extremely labile character of this compound is due to its chemical structure. It would be highly desirable and of a great practical importance to provide a stabilized prostacyclin or prostacyclin derivative suitable for therapeutic application (Science, December 20, 1976, p. 17).

According to this invention an inclusion complex of prostacyclin or a derivative thereof with a cyclodextrin is provided.

Cyclodextrins are cyclic molecules consisting of 6, 7 or 8 glucopyranose units forming α - 1,4 - glucoside units. Structurally they are characterized by a special arrangement of the hydroxyl groups. All the secondary hydroxyls are situated on one edge of the ring, while all the primary hydroxyls are placed on the other edge of that ring. Therefore the outer surface of the ring is essentially hydrophylic which ensures that the cyclodextrins are water-soluble. On the other hand the inner surface of the rings has a hydrophobic character since in that part of the molecule only hydrogen atoms and glucosidal oxygen bridges are to be found. Consequently, if molecules which are less polar than water and the shape and size of which enables them to penetrate into the cave inside the cyclodextrin are added to an aqueous solution of cyclodextrins, a cyclodextrin inclusion complex is formed even in an aqueous solution. This method proved to be useful for the stabilization of numerous compounds. The method is also useful for the reduction of the volatility of certain compounds and for the protection of various materials against atmospheric oxidation. The ring consisting of 6 glucopyranose units is called α-cyclodextrin, that consisting of 7 units is called β-cyclodextrin and finally the ring having 8 glucopyranose units is called γ-cyclodextrin.

The object of this invention is to provide inclusion complexes of prostacyclin and prostacyclin derivatives with cyclodextrins. These complexes are sufficiently more stable and have a much more prolonged action than prostacyclin and its derivatives, and may be used as active ingredients of pharmaceutical compositions for clinical purpose. These compositions may be administered as intravenous injections or subligual tablets etc, to treat and prevent the above-mentioned thrombi. Since these complexes act very rapidly, their use in many instances may save lives.

The prostacyclin derivatives used in the complexes of this invention are for example esters, ethers or other functional or structural derivatives of natural prostacyclin. The preferred prostacyclin derivatives are C_{1-10} alkyl, phenyl or phen-C_{1-10}-alkyl ethers or esters, wherein the phenyl group or residue optionally is substituted by one or more of the following groups: halogen, hydroxy, nitro, C_{1-6}-alkoxy, amino, C_{1-6}-alkyl-amino, C_{1-6}-dialkylamino group; or a functional or structural derivative of prostacyclin, such as a 15-epi- or dihydro-derivative thereof, and the above-exemplified ethers and esters of these derivatives.

Prostacyclin and its derivatives can e.g. be prepared according to the methods described in the following publications: Tetr. Let. *30*, 26-27 (1977); J. Am. Chem. Soc. *99*, 2006 (1977).

According to this invention the inclusion complexes of prostacyclin derivatives (or of prostacyclin) with cyclodextrin are prepared by contacting the corresponding prostacyclin or derivative, such as natural prostacyclin or an ether, ester or another functional or structural derivative thereof, preferably in organic solvent solution, with an aqueous solution of α-, β- or γ-cyclodextrin or a mixture of any of these and isolating the complex formed from the solution,

if desired after eliminating any organic solvent from the mixture suitably by lyophilization or by crystallisation at a low temperature.

The physiologically active inclusion complexes prepared as described above may be transformed into conventional pharmaceutical compositions in a manner known *per se*, by formulation with a pharmaceutically acceptable carrier or excipient. These compositions thus comprise the inclusion complexes of this invention in association with conventional excipients, diluents, ion-strength regulators and/or other additives and may be administered as injection and infusion solutions, tablets for oral or sublingual administration, capsules, dragees or powder ampoules.

As a cyclodextrin α-, β- or γ-cyclodextrin or their mixtures can equally well be used.

The prostacyclin or its derivative may be dissolved in various water-miscible, water-immiscible, or partially water-miscible solvents. For this purpose for example ethers, such as diethyl ether, tetrahydrofurane or dioxane; ketones, e.g. acetone; sulphoxides, e.g. dimethyl sulphoxide; and amides alkylated on the nitrogen atom, such as dimethyl formamide can be used. The mixtures of two or more of the solvents listed above may also be employed.

The aqueous cyclodextrin solutions preferably also contain various inorganic or organic salts, or buffers which play a role in the stabilization and adjustment of the pH and ion strength of the solution.

The invention will now be illustrated in greater detail in the following specific Examples, which are given for illustration and not limitation of our invention.

Example 1

To 1 ml. of a pH 8 phosphate-buffer solution 6 ml. of distilled water are added. The solution is heated up to 30 C° and 190 mg. (water-content: 14%) of β-cyclodextrin are added. When the dissolution is completed, 8 mg. of PGI_2 in 31 ml. of ether are added to the solution within one hour, with stirring. The ether vapour is eliminated while stirring under a slightly reduced pressure. When the ether vapour has been entirely eliminated the solution is allowed to cool to room temperature, under continuous stirring, in about one hour. The solution is then frozen and lyophilized in a conventional manner. 182 mg of a dry prostacyclin - β - cyclodextrin complex are obtained as a white powder, which is readily soluble in water.

Example 2

10 mg. of prostacyclin methyl ester are dissolved in 2 ml. of diethyl ether. Separately 200 mg. of anhydrous β-cyclodextrin are dissolved in a mixture of 1 ml. of a pH 8 buffer and 5.5 ml. of distilled water at 30° C and the above prostacyclin methyl ester solution is added to the solution obtained. The mixture is stirred for one hour, under a slightly reduced pressure and is then shaken on a shaker for further two hours at room temperature. The solution is frozen and lyophilized. 198 mg. of prostacyclin methyl ester β - cyclodextrin complex are obtained as an amorphous, water-soluble powder, which decomposes upon heating without melting.

Example 3

Capsule containing 2.5 mg. of active ingredient:—

Composition per capsule

PGI_2-β-CD complex (containing 5% of active ingredient)	50 mg.
Colloidal silicic acid	9 mg.
Talc	5 mg.
Magnesium stearate	15 mg.
Lactose	20 mg.
Crystalline cellulose	40 mg.
Potato starch	86 mg.

The capsules are prepared as follows.

A powder mixture is prepared by dry granulation of a mixture containing the components described above in the stated proportions. The powder mixture is homogenized and encapsulated on a standard machine to give capsules of 225 mg.

Example 4

Tablet containing 2.5 mg. of active ingredient:

Composition per tablet

PGI_2-β-CD complex (containing 5% of active ingredient)	50 mg.
Amylopectin	10 mg.
Crystalline cellulose	60 mg.
Stearic acid	2 mg.
Talc	13 mg.
Potato starch	280 mg.

The preparation of the tablets is as follows:

Prostacyclin - β - cyclodextrin inclusion complex, amylopectin and microcrystalline cellulose together with a corresponding quantity of potato starch are thoroughly admixed in a homogenizing machine in the proportions given above. The stated proportion of stearic acid and talc are homogenized and passed through a No. 100 sieve. The fine powder obtained is admixed with the homogeneous powder mixture first prepared, and the mixture obtained is converted into tablets weighing 415 mg. each. If desired, the tablets obtained are transformed into dragees or coated tablets.

Example 5

Injection (lyophilized)

An aqueous solution of the PGI_2-β-CD complex prepared according to Example 2 is filled into ampoules, each ampoule containing 5 mg. of the complex having an active ingredient concentration of 5%. The ampoules are then lyophilized and subsequently sealed under nitrogen. Prior to administration the lyophilized powder is dissolved in a physiological sodium chloride solution up to the desired volume.

Example 6

Platelet aggregation inhibition test

The platelet aggregation inhibition tests were carried out in a Born aggregometer, on human blood. Aggregation was induced by 20 μmoles of ADP (adenosine triphosphate) or with 1.2 mmoles of arachidonic acid. The β - cyclodextrin - prostacyclin inclusion complex was added into the platelet suspension before the addition of the inductor. Tests were performed at 20°C.

Aggregation induced by arachidonic acid

A fresh aqueous solution of the prostacyclin - β - cyclodextrin inclusion complex having a prostacyclin concentration of 40 μg/ml. exerted no inhibiting

effect on the aggregation of platelets. When the same solution was administered after a 30 minutes standing at room temperature, a 20% inhibition was observed.

A solution containing the complex in a quantity corresponding to a prostacyclin concentration of 5μg/ml. gave total inhibition of the platelet aggregation even 30, 60 and 150 minutes after the preparation of the solution.

When an aqueous solution of a prostacyclin - β - cyclodextrin inclusion complex was allowed to stand at room temperature for two hours, it was able to induce a total (100%) inhibition even in a concentration of 1 μg/ml.

Aggregation induced by ADP

The prostacyclin - β - cyclodextrin inclusion complex was dissolved in water and the solution obtained was allowed to stand at room temperature for 4 hours. Thereafter the inhibition of the aggregation induced by ADP was observed. The following results were obtained:

Concentration of prostacyclin	Inhibition
10 ng/ml.	50%
100 ng/ml.	100%
1μg/ml.	100%
10μg/ml.	100%

Solutions containing 20 to 400 μg/ml. of prostacyclin induced a disaggregation of the thrombi formed under the influence of ADP or ristocetin. The same results were obtained, when the solutions were allowed to stand at room temperature for 120 minutes prior to use.

In view of the fact that prostacyclin alone decomposes in aqueous solutions within 4 minutes, the above results prove that the stability of prostacyclin is highly improved by preparing its cyclodextrin complex. The observation that the fresh solutions have no aggregation-inhibiting effect is due to the fact that the dissociation of the inclusion complexes require some time. From the pharmacological aspect this feature is very advantageous, since at the time of administration the PGI_2 concentration is not injuriously high, while later on the rapid dissociation of the inclusion complex ensures that the PGI_2 concentration reaches the biologically active level for a long time.

When PGI_2 alone is added to human blood, disaggregating activity can be observed for about 2 minutes. During this very short time interval the PGI_2 is entirely decomposed. In contrast to this observation, the inclusion complex of prostacyclin has a duration of activity of 10 minutes, i.e. it has a five-times more prolonged action than PGI_2.

A preparation of mesenteric artery was treated with an aqueous solution of a PGI_2-β-cyclodextrin inclusion complex. A provisional relaxing effect characteristic of prostacyclin could be observed. On a rat uterus preparation a contraction corresponding to 0.01 to 0.001 of that induced by $PGR_{2\alpha}$ was observed, when the preparation was treated with the inclusion complex. The contractions induced by electric shock on a rabbit ore artery were inhibited by the inclusion complex having an active ingredient concentration of 10 to 20 μg/ml. PGI_2 ethyl ester showed the same effect in a concentration of 0.2 to 0.4 μg/ml.

The effect of the salivary amylase on the cyclodextrins and their complexes has been investigated. The evaluation of the mode of action of this reaction is necessary to enable us to determine whether the rate of PGI_2 liberation is regulated by the dissociation of the complex or by the enzymatic hydrolysis of the cyclodextrin. Using our enzyme preparation, in a pH 6 phosphate buffer at 35°C the decomposition of soluble starch to reducing sugar is complete (100%). On the other hand, a measurable increase in the reducing ability of β-cyclodextrin appears only after 5 hours. Consequently, the conclusion can be drawn that the β-cyclodextrin is practically entirely resistant to the saliva. From this statement it follows that from a subligual tablet prostacyclin can be liberated only by the dissociation of the inclusion complex.

The complex prepared according to Example 2 was kept in a sealed ampoule for 5 days, at room temperature. The ampoule was then opened, the content of it was poured into a flask and a 500-fold volume of ether was added. The flask was sealed and the mixture was stirred at room temperature for 30 hours. Occasionally samples taken out of the reaction mixture were subjected to thin layer chromatography (Kieselgel F_{254} produced by Merck, using a 1:1 mixture of ethyl acetate and acetone as a solvent mixture and chromesulphuric acid for the development). Even after 30 hours only a spot corresponding to prostacyclin methyl ester was obtained ($R_f = 0.50$). No sign of decomposition was noticed, i.e. the complex was stable in an aprotic solution, at a neutral pH-value.

Investigating the stability of prostacyclin-ethyl ester - β - cyclodextrin prepared essentially following the procedure described in Example 2, the following results were obtained:

The half life for the decomposition of the inclusion complex amounts to 87 minutes in a concentration of 10 γ/ml, in an aqueous solution (pH = 6.8 to 7).

Under the same conditions the half life of prostacyclin ethyl ester itself is 11 minutes.

By oral administration cyclodextrins are not toxic. To CFY female rats having an average weight of 250 g, 500 mg. of β-cyclodextrin were administered by means of a gastric tube, in the form of a suspension in a 0.01% methyl cellulose solution. The test animals were observed for 34 days. It was found that the test animals were developing in the same way as the control animals; no sign of toxicity was noticeable.

LD_{50} (i.v.) for α-cyclodextrin = 1 g/kg. of body weight; LD_{50} (i.v.) for β-cyclodextrin = 0.788 g/kg of body weight on Sprague-Dawley rats (Amer. J. Pathol. *83*, 367 (1976)).

From the results of the above experiments it can be seen that an effective prostacyclin concentration of a prostacyclin - cyclodextrin inclusion complex is in the region of 0.1 to 1 μg/ml. Calculated for 5 lit. of blood, 0.1 to 5 mg of PGI_2 is required which means 2 to 100 mg. of a PGI_2 inclusion complex having a PGI_2 concentration of 5%. The intravenous injection com-

positions and subligual tablets preferably have an active ingredient content within the above range.

CLAIMS

1. An inclusion complex of prostacyclin or a derivative thereof with a cyclodextrin.

2. An inclusion complex as claimed in claim 1 wherein said cyclodextrin is α-cyclodextrin.

3. An inclusion complex as claimed in claim 1 wherein said cyclodextrin is β-cyclodextrin.

4. An inclusion complex as claimed in claim 1 wherein said cyclodextrin is γ-cyclodextrin.

5. An inclusion complex as claimed in claim 1 wherein said cyclodextrin is a mixture of two or more of α-, β- and γ-cyclodextrins.

6. An inclusion complex as claimed in any of the preceding claims wherein said derivative of prostacyclin is an ester or ether of prostacyclin, 15-epi-prostacyclin, dihydroprostacyclin or an ester or ether of either of these.

7. An inclusion complex as claimed in claim 6 wherein said ester or ether is a C_{1-10} alkyl, phenyl or phen-C_{1-10}-alkyl ether or ester, said phenyl group or residue being optionally substituted by one or more substituents selected from halogen, hydroxy, nitro, C_{1-6}-alkoxy, amino, C_{1-6}-alkylamino and di-C_{1-6} alkylamino.

8. An inclusion complex of prostacyclin with a cyclodextrin.

9. An inclusion complex of prostacyclin with β-cyclodextrin.

10. An inclusion complex of prostacyclin methyl ester with β-cyclodextrin.

11. An inclusion complex of prostacyclin ethyl ester with β-cyclodextrin.

12. An inclusion complex as claimed in claim 1, substantially as described herein.

13. A process for the preparation of an inclusion complex according to any of the preceding claims which comprises contacting an aqueous solution of α-, β- or γ-cyclodextrin or a mixture of any of these with prostacyclin or a derivative thereof, and isolating the prostacyclin- or prostacyclin derivative - cyclodextrin inclusion complex.

14. A process as claimed in claim 13 wherein said aqueous solution also comprises inorganic or organic salts.

15. A process as claimed in claim 14 wherein said inorganic salts comprise a phosphate buffer.

16. A process as claimed in any of claims 13-15 wherein said aqueous solution is contacted with an organic solvent solution of prostacyclin or a derivative thereof, the organic solvent is eliminated from the mixture so obtained and the inclusion complex is isolated from the remaining aqueous solution.

17. A process as claimed in any of claims 13-16 wherein the isolation of said inclusion complex is performed at a low temperature, by crystallization.

18. A process as claimed in any of claims 13-16 wherein the isolation of said complex is performed by lyophilization.

19. A process as claimed in claim 13, substantially as described in Examples 1 or 2 herein.

20. A pharmaceutical composition comprising a physiologically active inclusion complex according to any of claims 1-7 and a pharmaceutically acceptable carrier or excipient.

21. A pharmaceutical composition as claimed in claim 20, which comprises an inclusion complex according to any of claims 8-11.

22. A pharmaceutical composition as claimed in claim 20 or 21 wherein said inclusion complex contains 0.1 to 5 mg of prostacyclin in each unit dose.

23. A pharmaceutical composition as claimed in any of claims 20-22, substantially as illustrated in Examples 3, 4 or 5.

24. Use of the complex as claimed in any of claims 1-12 for modifying platelet aggregation in a human patient.

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